

Metabolism of Benzo(a)pyrene and DNA
(Deoxy-Ribonucleic Acid) Adduct Formation in
Cultured Human Epidermal Keratinocytes

Columbia Univ., New York

Prepared for

Environmental Protection Agency
Research Triangle Park, NC

1981

U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA (Please read Instructions on the reverse before complete)		
1. REPORT NO. EPA-600/J-81-610	2. JOURNAL ARTICLE	3. PB83-164129
4. TITLE AND SUBTITLE JOURNAL ARTICLE: Metabolism of benzo[a]pyrene and DNA adduct formation in cultured human epidermal keratinocytes		5. REPORT DATE 1981
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) G. Theall, M. Eisinger and D. Grunberger		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Cancer Center/Institute of Cancer Research, Depts. of Biochemistry & PH Division of Environmental Sciences, Col. Univ. Coll. of Physicians & Surg., New York, NY and Sloan Kettering Cancer Center, New York		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. Grant No.-CR 807282
12. SPONSORING AGENCY NAME AND ADDRESS US Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA/600/11
15. SUPPLEMENTARY NOTES Carcinogenesis, Vol. 2, No. 7, pp. 581-587, 1981		
16. ABSTRACT Cultured human epidermal cells which require no feeder layer were used to study metabolism of benzo[a]pyrene (BP) and DNA adduct formation. The cultures were prepared from a single cell suspension and maintained at a pH of 5.9-6.2. At 2 μ M BP some cell toxicity was observed, and substantial cell death occurred at 4 μ M BP. The metabolism and DNA binding of BP were followed from 6 to 48 h of incubation. high pressure liquid chromatography (h.p.l.c.) revealed that BP was metabolized into 9,10-diol,7,8-diol, quinones, phenols and tetraols of BP. The DNA binding levels increased linearly up to 28 h of incubation. At 18 h, the level of DNA binding at 0.4 μ M BP was 1.5×10^{-6} mol BP/mol DNA and increased to 6.0×10^{-6} mol BP/mol DNA at a dose of 4 μ M BP. Analysis of the DNA adducts by h.p.l.c. indicates that the 2'-deoxy-N ² -(7,8,9,10-tetrahydro-7 β -,8 α -,9 α -trihydroxybenzo[a]pyrene-10-yl)guanosine was the predominant adduct formed in cells exposed to BP. The prevalence of the minor DNA adducts varies as a function of the source of the primary skin cells. These results confirm that human cells with no feeder layer metabolize BP and the resultant DNA damage is similar to that found in other mammalian systems.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161		
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

ATTENTION

AS NOTED IN THE NTIS ANNOUNCEMENT,
PORTIONS OF THIS REPORT ARE NOT LEGIBLE.
HOWEVER, IT IS THE BEST REPRODUCTION
AVAILABLE FROM THE COPY SENT TO NTIS.

Metabolism of benzo[a]pyrene and DNA adduct formation in cultured human epidermal keratinocytes

Gail Theall¹, Magdalena Eisinger² and Dezider Grunberger¹

¹Cancer Center/Institute of Cancer Research, Departments of Biochemistry and Public Health Division of Environmental Sciences, Columbia University College of Physicians and Surgeons, New York, NY 10032, and ²Memorial Sloan Kettering Cancer Center, New York, NY 10021, USA.

(Received on 17 February 1981; accepted on 13 May 1981)

Abstract

Cultured human epidermal cells which require no feeder layer were used to study metabolism of benzo[a]pyrene (BP) and DNA adduct formation. The cultures were prepared from a single cell suspension and maintained at a pH of 5.9–6.2. At 2 μ M BP some cell toxicity was observed, and substantial cell death occurred at 4 μ M BP. The metabolism and DNA binding of BP were followed from 6 to 48 h of incubation. High pressure liquid chromatography (h.p.l.c.) revealed that BP was metabolized into 9,10-diol, 7,8-diol, quinones, phenols and tetraols of BP. The DNA binding levels increased linearly up to 28 h of incubation. At 18 h, the level of DNA binding at 0.4 μ M BP was 1.5×10^{-6} mol BP/mol DNA and increased to 6.0×10^{-6} mol BP/mol DNA at a dose of 4 μ M BP. Analysis of the DNA adducts by h.p.l.c. indicates that the 2'-deoxy-N²-(7,8,9,10-tetrahydro-7 β -,8 α ,9 α -trihydroxybenzo[a]pyrene-10-yl) guanosine was the predominant adduct formed in cells exposed to BP. The prevalence of the minor DNA adducts varies as a function of the source of the primary skin cells. These results confirm that human cells with no feeder layer metabolize BP and the resultant DNA damage is similar to that found in other mammalian systems.

Introduction

The metabolism of benzo[a]pyrene (BP)* and its subsequent binding to DNA has been extensively studied in cell cultures (1–3), in whole animals (4,5), and microsomal systems (6–8). As BP is a prevalent industrial pollutant linked to human cancer (9,10), studies of its metabolism and DNA binding in human

cell cultures is important for our understanding of chemical carcinogenesis in man. BP, not mutagenic in itself, must be metabolized by the cytochrome P-450 enzyme system (11) into mutagenic metabolites. Many metabolites are formed but two predominate in DNA binding. These metabolites are the *anti* and *syn* isomers of 7,8-dihydrobenzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE). These epoxides bind primarily to guanine residues in nucleic acids and the relative levels of the adducts formed vary from cell system to system.

Human cells have been used to study BP metabolism and binding (16–20). In human bronchial explants, the only DNA adduct observed was that formed from reaction of the *anti* isomer of BPDE with deoxyguanosine (BPDE I-dG) (21), whereas in cultured human colon, both the *anti* and the *syn* adducts are present (22). Reinwald and Green (23) developed a human epidermal cell culture which required a feeder layer of irradiated 3T3 cells for growth. Taking into account the effect of the irradiated 3T3 cells, Parkinson and Newbold (24) showed that human epidermal cells are capable of metabolizing and binding BP. Kitano *et al.* (25) developed a human epidermal keratinocyte culture system which requires no feeder layer for growth. With this system, Kuroki *et al.* (26) demonstrated that different primary human cultures convert BP into different metabolites.

We chose to work with human epidermal keratinocytes because of normal contact of human skin with environmental contaminants. The cell culture system utilized was developed by Eisinger *et al.* (27,28). In this system, the keratinocytes at acidic pH attach to plastic dishes and grow to confluency in 6–8 days. They form structures of 11–13 cell layers after 18 days from initial seeding. We have investigated the effect of the dose of BP and incubation time on the metabolism and extent of DNA modification in five identically grown primary human epidermal keratinocyte cultures.

Materials and Methods

Chemicals

BP and alkaline phosphatase (*E. coli* Type III) were purchased from Sigma Chemical Co., St. Louis, MO, USA; venom phosphodiesterase, spleen phosphodiesterase, and deoxyribonuclease I were from Worthington Biochemical, Freehold, NJ, USA; *Neurospora crassa* endonuclease was from Miles Biochemicals, Elkhart, IN, USA; and trypsin from DIFCO Lab., Detroit, MI, USA. Generally labeled [³H]BP (40–65 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, IL, USA. Fetal calf serum was from Microbiological, St. Louis, MO, USA, and the media from Grand Island Biological Co., Grand Island, NY, USA.

*Abbreviations BP, benzo[a]pyrene; BPDE I, 7,8,9,10-tetrahydro-7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-benzo[a]pyrene; BPDE II, 7,8,9,10-tetrahydro-7 β ,8 α -dihydroxy-9 β ,10 β -epoxy-benzo[a]pyrene; BPDE I-dG, 2'-deoxy-N²-(7,8,9,10-tetrahydro-7 β ,8 α ,9 α -trihydroxybenzo[a]pyrene-10-yl)guanosine; h.p.l.c., high pressure liquid chromatography; tetraol, 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxybenzo[a]pyrene; 9,10-diol, *trans*-9,10-dihydro-9,10-dihydroxybenzo[a]pyrene; 7,8-diol, *trans*-7,8-dihydro-7,8-dihydroxybenzo[a]pyrene; 4,5-diol, *trans*-4,5-dihydro-4,5-dihydroxybenzo[a]pyrene.

Tissue culture

Split thickness human breast skin specimens obtained from surgery for breast reduction or cadaver skin shavings (3–4 h after death) were used. Cultures 681 and 679 were derived from skin of various areas of a 68 and 85-year-old male 3–4 h post-mortem. Cultures 662 and 740 were taken from surgically removed breast skin of two middle aged women. Culture 759 was from breast skin removed from a woman with carcinoma. The tissue culture was similar to the procedure described previously by Eisinger *et al.* (27). Skin samples were washed in minimal essential medium with Earle's salts, containing per ml 1000 units of penicillin, 1 mg of streptomycin and 2.5 µg of fungizone for 30 min, followed by two washes of 10 min each in the same medium and by two additional washes in minimal essential medium with 1/10 the concentration of antibiotics. The skin was then cut with scissors into approximately 0.2 x 0.5 cm pieces. After washing in 0.02% EDTA, the pieces were transferred to 0.25% trypsin at 4 °C for 12–15 h. The trypsin was replaced by minimal essential medium, containing non-essential amino acids, 2 mM L-glutamine, penicillin, streptomycin, and 5% fetal calf serum, and the epidermis of each piece was detached from the dermis with forceps. Isolated epidermal pieces were pooled in a trypsin/EDTA solution and a single cell suspension was obtained by gentle pipetting. Cellular clumps were removed by filtration through two to three layers of sterile gauze and single cells were collected into fetal calf serum. The cells were concentrated by centrifugation (10 min x 180 g) and resuspended in Eagle's minimal essential medium plus non-essential amino acids, 2 mM L-glutamine, hydrocortisone 0.4 µg/ml, 10% fetal bovine serum, penicillin and streptomycin. The pH was adjusted to 5.9–6.2 with 1 M HCl and cells were seeded at a density of 2×10^5 cells per cm². Incubation was at 36 °C in water-saturated air containing 5% CO₂.

Treatment of cultures with BP

Confluent cultures, 2 weeks after seeding, were gently trypsinized to remove cornified cells and fed with complete medium 24 h prior to the addition of BP. Concentration of BP and time of treatment is indicated in the Results. To assess the toxicity, 0.5 µg, 1 µg, 2 µg, 5 µg/ml BP were added to the cultures. Cell viability was evaluated using a trypan blue dye exclusion method (29).

BP metabolites

The culture media (1 ml) were extracted with ethyl-acetate three times, dried over sodium sulfate, evaporated to dryness and dissolved in 1 ml methanol. The radioactivity in the media, in the water and ethyl-acetate extracts was measured. The ethyl-acetate extracted material was analyzed by high pressure liquid chromatography (h.p.l.c.) on a DuPont 850 instrument using a Waters µBondapak C₁₈ column. The column was developed using a linear gradient of 40–100% methanol in water over 100 min. The flow rate was 1

ml/min. U.v. absorbing markers were 9,10-diol, 7,8-diol from the Cancer Research Program of the NCI, Division of Cancer Cause and Prevention, Bethesda, MD, USA, and 7,8,9,10-tetraol and BP. Fractions of 1 ml were collected and assayed by scintillation counting on an LKB 1215 Rackbeta liquid scintillation counter.

DNA binding and adduct analysis

The cells were digested with 0.1 % SDS, 10 mM Tris-HCl (pH 7.9), 10 mM EDTA and 200 µg/ml proteinase K for 2 h at 37 °C. The lysate was extracted three times with phenol:chloroform:8-OH quinoline (1:1:0.001) and precipitated with 2.5 volumes of –20 °C ethanol. The nucleic acid was treated with RNase A (25 µg/ml) for 2 h at 37 °C, pronase (10 µg/ml) for 1 h, and then phenol extracted and precipitated. Binding levels were determined by quantitation of DNA by u.v. spectroscopy and BP bound by levels of radioactivity as determined by scintillation counting. The DNA was enzymatically hydrolyzed as reported by Yamasaki *et al.* (30).

The hydrolyzed DNA samples were chromatographed on a Sephadex LH-20 column using a stepwise elution of water and 80% methanol in water. Characterization of the BP–nucleoside products were performed by h.p.l.c. using a Waters µBondapak C₁₈ column developed with a 35–60% non-linear methanol-water gradient at 50 °C at 1 ml/min flow rate over 100 min. Markers included u.v. detectable BPDE I–dG, BPDE II–dG, and BPDE I tetraols and the presumptive radio labeled 9-hydroxy-4,5-oxide BP–dG adduct. All h.p.l.c. profiles were repeated three times.

Results

Cell toxicity

The effect of BP on the human epidermal keratinocytes was explored on 2-week-old cultures which were composed predominantly of basal cells and a minor population of keratinizing cells. The sheets, when viewed by light microscopy, show a regular pavement-like mosaic structure. When viewed by electron microscopy, they were shown to possess desmosomes and tonofilaments. In addition to keratinocytes, a minor population of the cells are melanocytes with well-developed melanosomes and melanin granules. After 24 h incubation with increasing doses of BP, the cultures did not show noticeable cytotoxic changes when viewed by light microscopy. However, when cells after 24 h treatment with BP were allowed to grow for an additional 5 days and the numbers of viable cells were counted, it was found that in 2 µM BP 65% and in 20 µM BP only 18% of the cells survived (see Figure 1). The growth pattern of subcultured cells treated with 2 µM BP did not differ from the control. However, when cells treated with 4 and 8 µM BP were trypsinized and seeded, growth of only small islands of epidermal keratinocytes was observed. The growth of cells treated with 20 µM BP

was completely inhibited. Different primary cultures display a slight variance in the degree of toxicity.

Relationship of BP metabolism to DNA binding levels

In order to investigate the effect of metabolism of BP on the level of DNA modification, the supernatants from cells treated with BP were used for metabolic studies and the cells were used for DNA analysis. The cultures were 15-day-old primary epidermal cells in an exponential stage of growth. The media from tissue cultured cells were removed 6, 18, 28 and 48 h after addition of $1.3 \mu\text{M}$ BP. Figure 2 relates DNA binding and the levels of BP and its metabolites in culture 681 as a function of the BP incubation time. The concentration of BP decreases steadily with time, and by 28 h, only 5% of the BP is not metabolized. The BP is converted into water-soluble and ethyl-acetate extractable metabolites. As the concentration of BP decreases, the level of water-soluble products increases. The ethyl-acetate metabolites, however, reach a peak level at 18 h and begin to decrease slightly with increased incubation time. This, presumably, is due to their further detoxification. Concomitantly, the binding of BP residues to DNA increases linearly during the first 28 h to a maximal level of 9.1×10^{-6} mol BP/mol DNA. This level is retained through 48 h.

The relationship between the BP dose and its metabolites and DNA binding in culture 759 is seen in Figure 3. Up to a dose of $2 \mu\text{M}$, the cells metabolize 98% of the BP after 18 h. At a dose of $4 \mu\text{M}$, 90% has been metabolized. The water-soluble, as well as the ethyl-acetate extractable, metabolites increase throughout the entire dose range tested. The binding of BP residues to DNA also increases.

Ethyl-acetate extractable metabolites

The duration of BP exposure to the culture makes a significant difference in the levels of the metabolites found in the culture media. A detailed analysis of total ethyl-acetate metabolites presented in Figures 2 and 3 was performed by h.p.l.c. Figure 4 shows the elution profiles of the ethyl-acetate metabolites corresponding to 6 (A), 18 (B), and 48 (C) h incubations of culture 781 with $1.3 \mu\text{M}$ BP. The metabolites have been identified as 7,8,9,10-tetrahydrobenzo[a]pyrene (tetraol), *trans*-9,10-dihydro-9,10-dihydroxybenzo[a]pyrene (9,10-diol), *trans*-7,8-dihydro-7,8-dihydroxybenzo[a]pyrene (7,8-diol), quinones and phenols. A series of peaks eluting in the initial fractions have not been identified. The quinones and the phenols of BP are mixtures which are not resolved in this chromatographic system. As can be seen in Figure 4A, by 6 h a substantial amount of 9,10-diol and 7,8-diol have been formed. At 18 h (B), there is more 9,10-diol and tetraol, and only a slightly elevated level of 7,8-diol. The ratio of 7,8-diol to 9,10-diol is two-fold lower than at 6 h. At 48 h (C), the only ethyl-acetate soluble metabolites remaining are tetraols, triols and a substantial level of unidentified polar products.

The investigation of the possible interindividual

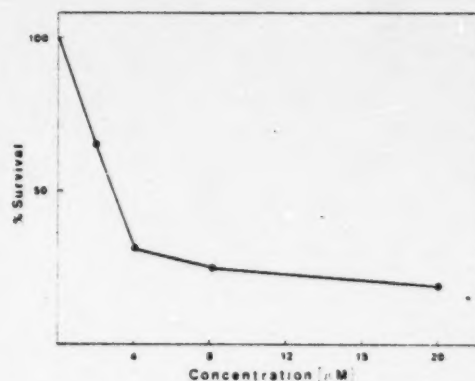


Fig. 1. Number of viable human epidermal keratinocytes 5 days after a 24 h incubation with different doses of BP.

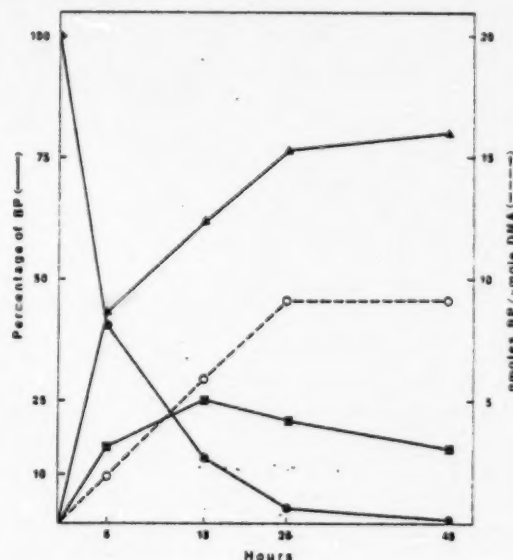


Fig. 2. The correlation of BP metabolism and DNA binding with incubation time. The levels of water-soluble metabolites (▲—▲), ethyl-acetate extractable metabolites (■—■), BP (●—●) are expressed as the percentage of the initial dose of $1.3 \mu\text{M}$ [^3H]BP. The total BP-DNA binding levels (○—○) are expressed as pmol BP bound/ μmol DNA.

variations in ethyl-acetate extractable metabolites of BP was performed with several different primary cultures. Figure 5 represents h.p.l.c. profiles of BP metabolites from cultures 681, 662 and 679. All three cultures generated essentially the same metabolites. However, there are differences in the relative levels of individual metabolites especially for the 9,10-diol to 7,8-diol ratio.

BP-DNA adduct formation

A detailed analysis of the DNA adducts was also performed on the five different cultures. The DNA was hydrolyzed and chromatographed on a LH-20 Sephadex column (not shown). The methanol eluant contained 60% of the BP bound DNA in each culture. Figure 6

Table I

Binding of BP to DNA in various primary human epidermal cultures and the relative abundance of individual peaks eluted by h.p.l.c.

Culture	Dose (μM)	Incubation (h)	Binding pmol BP/ μmol DNA	Relative abundance of individual peaks (%) ^a				
				Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
681 ^b	1.3	18	5.9	4.2	2.0	2.5	74.5	6.0
662 ^b	1.2	21	6.9	9.6	8.0	16.1	49.2	5.8
679 ^b	1.2	24	2.9	12.1	20.5	23.5	24.5	5.7
740 ^c	1.2	18	6.8	14.0	9.9	21.0	42.2	3.4
759 ^c	1.3	18	3.6	7.8	6.0	14.4	52.5	5.2

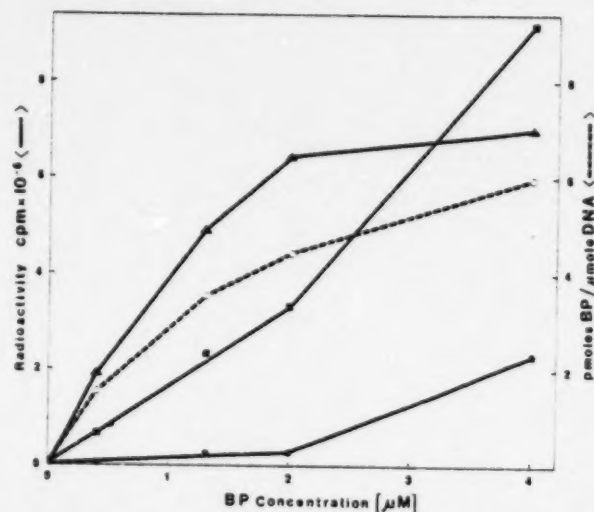
^a Percentage is based on total c.p.m. eluting from the h.p.l.c.^b From Figure 6.^c Not shown in Figure 6.

Fig. 3. The correlation of the increasing BP concentration with its metabolism and DNA binding. The levels of water-soluble metabolites (▲—▲), ethyl-acetate extractable metabolites (■—■), and BP (●—●) expressed as radioactivity. The BP-DNA binding levels (○—○) are expressed as pmol BP/ μmol DNA. Incubation time was 18 h.

shows three h.p.l.c. profiles of the methanol elution products. When culture 681 was incubated with BP for various lengths of time and at various doses, qualitatively, the same elution profile was observed (Figure 6A). Also, dose increases with culture 759 did not affect the h.p.l.c. elution pattern. The predominant adduct (peak 4) is identified as the BPDE I bound to deoxyguanosine at the N² position (BPDE I-dG). This adduct has been seen in human bronchial explants (21), colon explant (22), lung cells (31) and human mammary cells (20). However, when cultures derived from different sources were used, the BP-DNA adduct profiles were surprisingly different. Figure 6B and C are the h.p.l.c. profiles from cultures 662 and 679, respectively. Peak 1 elutes in the region of the BP tetraol marker. Peak 2 is in the general position that a BPDE-deoxycytosine adduct would be expected; however, identification cannot be made without proper markers.

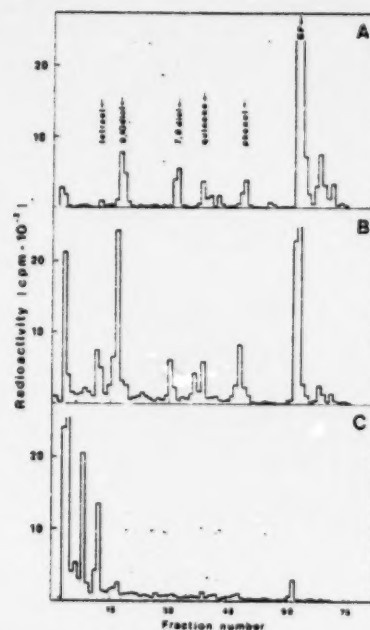


Fig. 4. H.p.l.c. profiles of ethyl-acetate extracted BP metabolites. Culture 681 was incubated with 1.3 μM BP for 6 h (A); 18 h (B); and 48 h (C). The arrows indicate the positions of the u.v. markers.

Peak 3 coelutes with a marker thought to be an adduct of deoxyguanosine and 9-hydroxy-4-5-oxide BP seen extensively in microsomal systems (32–34). The marker which we used to identify this product was prepared according to Thompson *et al.* (32). Since the structure of this adduct has not been determined yet, complete characterization of the product is required. Peak 5 coelutes with BPDE II-dG marker.

A comparison of the three samples displays a significant difference in not only the type of BP-DNA adducts formed but also in the relative levels of these adducts (Figure 6). Table I illustrates the relative abundance of individual h.p.l.c. elution products in five different cultures. Peak 4 BPDE I-dG, represents the major DNA adduct in all of the cultures; however, the level varies from 25% to 75% of the LH-20 methanol

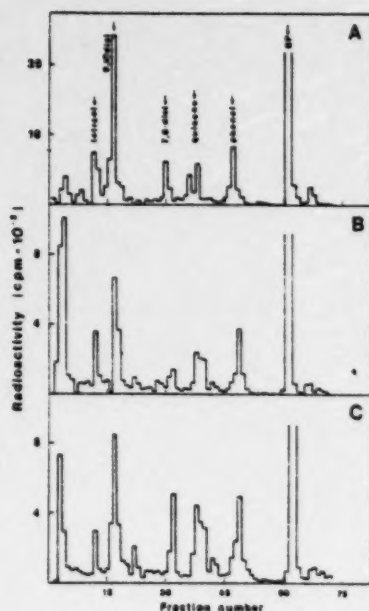


Fig. 5. H.p.l.c. profiles of ethyl-acetate extracted metabolites from various cultures. Culture 681 was incubated 18 h with $1.3 \mu\text{M}$ BP (A). Culture 662 was incubated 21 h with $1.2 \mu\text{M}$ BP (B). Culture 679 was incubated 24 h with $1.2 \mu\text{M}$ BP (C). The arrows indicate the positions of the u.v. markers.

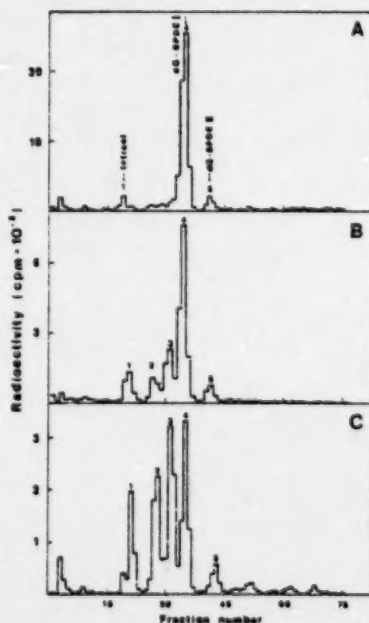


Fig. 6. H.p.l.c. profiles of DNA digests eluted in the methanol region of LH-20 Sephadex from culture 681 (A), culture 662 (B) and culture 679 (C).

eluant. The BPDE II-dG, peak 5, levels remain relatively constant at 5% of the total DNA adducts.

Peaks 1, 2 and 3 vary considerably. Peak 3 is present in significant levels in four of the five cultures. There is no apparent relationship between the levels of total binding and the types of BP-DNA adducts formed in the various cultures.

Discussion

Human epidermal keratinocytes are capable of metabolizing BP at a substantial rate. In the keratinocyte cultures, the 9,10-diol and the 7,8-diol were released into the media and the levels varied significantly with the incubation time and dose. When cultures derived from several different individuals were incubated with BP under similar conditions, they converted BP into the same metabolites at comparable levels. Although interindividual differences in the ratios of the diols were observed, the variance was no greater than the range of the ratios seen in one culture. This result is not in complete agreement with Kuroki *et al.* (26) who observed significantly different metabolic abilities of three primary cultures. This disparity could be the result of different tissue culture conditions, different doses of BP, as well as different sources of the primary cultures used. The results of Parkinson and Newbold (24) observing no more than a 2–3 fold difference in the metabolism of BP in keratinocytes from six patients are more in accord with our observations.

Although the cultures tested show similar metabolic activity by h.p.l.c. analysis, remarkable differences are observed in DNA adduct formation. However, the major adduct observed in all human epidermal cell cultures is BPDE I-dG. The BPDE II-dG adduct is a minor contributor to overall DNA binding. Unlike the hamster embryo cell system (35), the relationship of BPDE I-dG to BPDE II-dG adducts remained constant as the exposure time and BP dose was altered. The appearance of peak 3 in Figure 6B and C could represent an adduct formed by binding of 9-hydroxy-4,5-oxide BP to deoxyguanosine residues. Although there have been reports that BP binds DNA via an epoxidation of 9-hydroxy-BP. (32,33) to yield a 9-hydroxy-4,5-oxide BP-dG adduct, the structural characterization of this product has not been determined yet. This adduct, although it has been observed *in vitro* and in *Salmonella typhimurium* test systems (34), has not previously been reported in human cells.

There are several possible explanations for the difference in DNA adduct formation among various cultures. Epoxide hydrolase, an essential enzyme in the cytochrome P-450 system, has been shown by Guenther and Oesch (36) to have an important role in DNA adduct formation *in vitro*. High levels of epoxide hydrolase favor binding of 7,8-diol products to DNA whereas low levels of the enzyme result in the preferential formation of 9-hydroxy-benzo[a]pyrene-DNA adducts. Thus, the observed interindividual variation in BP-DNA adduct formation can be the

result of different levels of epoxide hydrolase activity in different cultures as was found by Oesch *et al.* (37) in human fibroblasts. Another reason for the variation in DNA adducts could be an altered ability of the cells to detoxify the BP metabolites by formation of glucuronide, glutathione, sulfate or other conjugates (38-40). A two-fold variation in glutathione S-transferase activity in human fibroblasts has been observed (37). It has also been demonstrated that cellular nucleophiles can selectively effect the *in vitro* binding of BP metabolites to nuclear DNA (41). Differences in detoxification enzymes and potential interindividual variations in cellular nucleophiles may result in varied DNA adduct formation by the selective removal of BP metabolites. Finally, the possibility that DNA repair is a significant factor in the removal of specific BP-DNA adducts in different cultures is less likely due to the short duration of these experiments.

Thus, quantitative relationships between mechanisms of activation and detoxification may have a primary role in the determination of the interindividual variation in DNA adduct formation. Therefore, only a better understanding of these complex processes could help to explain the variability of human cells to bind carcinogens and would in addition provide insights into the altered susceptibilities to carcinogens seen in the human population.

Acknowledgements

This investigation was supported by Grants EPA CR-807282 awarded by U.S. Environmental Protection Agency; CA 13696 awarded by the National Cancer Institute, DHHH; and PCM 7911783 awarded by the National Science Foundation.

References

1. Duncan, M., Brookes, P., and Dipple, A. (1969), Metabolism and binding to cellular macromolecules of a series of hydrocarbons by mouse embryo cells in culture, *Int. J. Cancer*, **4**, 813-819.
2. Brown, H.S., Jeffrey, A.M., and Weinstein, I.B. (1979), Formation of DNA adducts in 10T½ mouse embryo fibroblasts incubated with benzo[a]pyrene or dihydrodiol oxide derivatives, *Cancer Res.*, **39**, 1673-1677.
3. Ivanovic, V., Geacintov, N.E., Yamasaki, H., and Weinstein, I.B. (1978), DNA and RNA adducts formed in hamster embryo cell cultures exposed to benzo[a]pyrene, *Biochemistry*, **17**, 1597-1603.
4. Koreeda, M., Moore, P.D., Wislocki, P.G., Levin, W., Conney, A.H., Yagi, H., and Jerina, D.M. (1978), Binding of benzo[a]pyrene 7,8-diol-9,10-epoxides to DNA, RNA, and protein of mouse skin occurs with high stereoselectivity, *Science*, **199**, 778-781.
5. Brookes, P., and Lawley, P.D. (1964), Evidence for the binding of polynuclear hydrocarbons and nucleic acids of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to DNA, *Nature (Lond.)*, **202**, 781-784.
6. Pelkonen, O., Boobis, A.R., Yagi, H., Jerina, D.M., and Nebert, D.W. (1977), Tentative identification of benzo[a]pyrene metabolite-nucleoside complexes produced *in vitro* by mouse liver microsomes, *Mol. Pharmacol.*, **14**, 306-322.
7. Yang, S.K., Roller, P.P., and Gelboin, H.V. (1977), Enzymatic mechanism of benzo[a]pyrene conversion to phenols and diols and an improved h.p.l.c. separation of benzo[a]pyrene derivatives, *Biochemistry*, **16**, 3680-3687.
8. Selkirk, J.K., Croy, R.G., and Gelboin, H.V. (1974), Benzo[a]pyrene metabolites: efficient and rapid separation by high pressure liquid chromatography, *Science*, **184**, 169-170.
9. Hammond, E.C., Selikoff, I.J., Lawther, P.L., and Seidman, H. (1976), Inhalation of benzo[a]pyrene and cancer in man, *Ann. N.Y. Acad. Sci.*, **271**, 116-124.
10. I.A.R.C. (1973), *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 3, International Agency for Research on Cancer, Lyon, France, pp. 91-137.
11. Gelboin, H.V. (1967), Carcinogens, enzyme induction and gene action, *Adv. Cancer Res.*, **10**, 1-81.
12. Jennette, K.W., Jeffrey, A.M., Blobstein, S.H., Beland, F.A., Harvey, R.G., and Weinstein, I.B. (1977), Nucleoside adducts from the *in vitro* reaction of benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide or benzo[a]pyrene-4,5-oxide with nucleic acids, *Biochemistry*, **16**, 932-938.
13. Sims, P., Grover, P.L., Swaisland, A., Pai, K., and Hewer, A. (1974), Metabolic activation of benzo[a]pyrene proceeds by a diol-epoxide, *Nature (Lond.)*, **252**, 326-327.
14. Grover, P.L., Hewer, A., and Sims, P. (1972), Formation of K-region-epoxides as microsomal metabolites of benzo[a]pyrene, *Biochem. Pharmacol.*, **21**, 2713-2726.
15. King, H.W.S., Thompson, M.H., and Brookes, P. (1975), The benzo[a]pyrene deoxyribonucleoside products isolated from DNA after metabolism of benzo[a]pyrene by rat liver microsomes in the presence of DNA, *Cancer Res.*, **35**, 1263-1269.
16. Harris, C.C., Hsu, J.C., Stoner, G.D., Trump, B.F., and Selkirk, J.K. (1978), Human pulmonary alveolar macrophages metabolize benzo[a]pyrene to proximate and ultimate mutagens, *Nature (Lond.)*, **272**, 633-634.
17. Stoner, G.D., Harris, C.C., Autrup, H., Trump, B.F., Kingsbury, E.W., and Myers, G.A. (1978), Explant culture of human peripheral lung. I. Metabolism of benzo[a]pyrene, *Lab. Invest.*, **38**, 685-692.
18. Cohen, G.M., Mehta, R., Meredith-Brown, M. (1979), Large interindividual variations in metabolism of benzo[a]pyrene by peripheral lung tissue from lung cancer patients, *Int. J. Cancer*, **24**, 129-133.
19. Huberman, E., and Sachs, L. (1973), Metabolism of the carcinogenic hydrocarbon benzo[a]pyrene in human fibroblasts and epithelial cells, *Int. J. Cancer*, **11**, 412-418.
20. MacNicol, A.D., Easty, G.C., Neville, A.M., Grover, P.L., and Sims, P. (1980), Metabolism and activation of carcinogenic polycyclic hydrocarbons by human mammary cells, *Biochem. Biophys. Res. Commun.*, **95**, 1599-1606.
21. Jeffrey, A.M., Weinstein, I.B., Jennette, K.W., Grzeskowiak, K., Nakanishi, K., Harvey, R.G., Autrup, H., and Harris, C. (1977), Structures of benzo[a]pyrene-nucleic acid adducts formed in human and bovine bronchial explants, *Nature (Lond.)*, **269**, 348-350.
22. Autrup, H., Harris, C.C., Trump, B.F., and Jeffrey, A.M. (1978), Metabolism of benzo[a]pyrene and identification of the major benzo[a]pyrene-DNA adducts in cultured human colon, *Cancer Res.*, **38**, 3689-3696.
23. Reinwald, J.G., and Green, H. (1975), Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells, *Cell*, **6**, 331-343.
24. Parkinson, E.K., and Newbold, R.F. (1980), Benzo[a]pyrene metabolism and DNA adduct formation in serially cultivated strains of human epidermal keratinocytes, *Int. J. Cancer*, **26**, 289-299.
25. Kitano, Y., and Hata, S. (1972), The human epidermal cells infected with herpes simplex virus *in vitro*, *Arch. Derm. Forsch.*, **245**, 203-210.
26. Kuroki, T., Nemoto, N., and Kitano, Y. (1980), Metabolism of benzo[a]pyrene in human epidermal keratinocytes in culture, *Carcinogenesis*, **1**, 559-565.
27. Eisinger, M., Lee, J.S., Hefton, J.M., Darzynkiewicz, Z., Chiao, J.W., and deHarven, E. (1979), Human epidermal cell cultures: growth and differentiation in the absence of dermal components or medium supplements, *Proc. Natl. Acad. Sci. USA*, **76**, 5340-5344.
28. Eisinger, M., Monden, M., Raaf, J.H., and Fortner, J.G. (1980), Wound coverage by a sheet of epidermal cells grown *in vitro* from dispersed single cell preparations, *Surgery*, **88**, 287-293.
29. Phillips, H.J. (1973), Dye exclusion tests for cell viability, in Kruse, F.P., and Patterson, M.K. (eds.), *Tissue Culture Methods and Applications*, Academic Press, NY, pp. 406-408.
30. Yamasaki, H., Pulkrabek, P., Grunberger, D., and

- Weinstein, I.B. (1977), Differential excision from DNA of the C-8 and N² guanosine adducts of N-acetyl-2-aminofluorene by single-strand specific endonucleases, *Cancer Res.*, **37**, 3756-3760.
31. Feldman, G., Remson, J., Shinohara, K., and Cerutti, P. (1978), Excisability and persistence of benzo[a]pyrene DNA adducts in epitheloid human lung cells, *Nature (Lond.)*, **274**, 796-798.
32. Thompson, M.H., King, H.W.S., Osborne, M.R., and Brookes, P. (1976), Rat liver microsome-mediated binding of benzo[a]pyrene metabolites to DNA, *Int. J. Cancer*, **17**, 270-274.
33. King, H.W.S., Thompson, M.H., and Brookes, P. (1976), The role of 9-hydroxybenzo[a]pyrene in the microsomes mediated binding of benzo[a]pyrene to DNA, *Int. J. Cancer*, **18**, 339-344.
34. Santella, R.M., Grunberger, D., and Weinstein, I.B. (1979), DNA - benzo[a]pyrene adducts formed in a *Salmonella typhimurium* mutagenesis assay system, *Mutat. Res.*, **61**, 181-189.
35. Baird, W.M., and Diamond, L. (1977), The nature of benzo[a]pyrene-DNA adducts formed in hamster embryo cells depends on the length of time of exposure to benzo[a]pyrene, *Biochem. Biophys. Res. Commun.*, **77**, 162-167.
36. Guenther, T.M., and Oesch, F. (1981), The effects of modulation of microsomal epoxide hydrolase activity on microsome-catalyzed activation of benzo[a]pyrene and its covalent binding to DNA, *Cancer Lett.*, **11**, 175-183.
37. Oesch, F., Tegtmeier, F., Kohl, F.V., Rudiger, H., and Glatt, H.R. (1980), Interindividual comparison of epoxide hydratase and glutathione S-transferase activities in cultured human fibroblasts, *Carcinogenesis*, **1**, 305-309.
38. Nemoto, N., Herakawa, T., and Takayama, S. (1978), Glucuronidation of benzo[a]pyrene in hamster embryo cells, *Chem.-Biol. Interactions*, **22**, 1-14.
39. Nemoto, N., Takayama, S., and Gelboin, H.V. (1978), Sulfate conjugation of benzo[a]pyrene metabolites and derivatives, *Chem.-Biol. Interactions*, **23**, 19-30.
40. Cooper, C.S., Hewer, A., Ribeiro, O., Grover, P.L., and Sims, P. (1980), The enzyme-catalyzed conversion of *anti* benzo[a]pyrene-7,8-diol-9,10-oxide into a glutathione conjugate, *Carcinogenesis*, **1**, 1075-1080.
41. Guenther, T.M., Jernstrom, B., and Orrenius, S. (1980), On the effect of cellular nucleophiles on the binding of metabolites of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene and 9-hydroxybenzo[a]pyrene to nuclear DNA, *Carcinogenesis*, **1**, 407-418.